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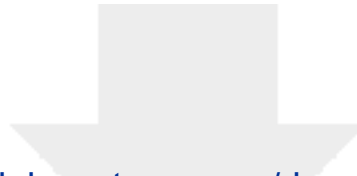
Interindividual responses of appetite to acute exercise: a replicated crossover study --Manuscript Draft--

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Dear Dr. Larson-Meyer,

RE: MSSE-D-17-00978

08/11/2017

We would like to thank the reviewers for giving their time to carefully examine our manuscript. Our research team are delighted to be given the opportunity to revise our manuscript for additional consideration by *Medicine and Science in Sports and Exercise*. Please find below a list of point-by-point responses to the comments raised by the reviewers. For clarity, changes to the manuscript have been highlighted in yellow. We hope that we have interpreted these comments accurately and that our responses and manuscript modifications are satisfactory.

We look forward to hearing about our paper in due course.

Yours sincerely,

A handwritten signature in black ink that reads "David Stensel." The signature is written in a cursive, flowing style.

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Reviewer one:

Comment #1: It was a pleasure to review this carefully prepared manuscript. The related concepts of personalised treatment and inter-individual differences (i.e. identifying responders versus non-responders) are currently very topical in the literature and regularly discussed in many papers in the literature and this journal in particular, both in relation to nutrition and the many other disciplines within the remit of MSSE. However, few studies include the necessary measurements to properly support such discussion. Within this context, the experimental design described here has been well-conceived and is precisely what is required both to advance understanding of individual differences in appetite regulation and also to show how individual differences can and should be studied. Beyond the design, all necessary details of the methodology are reported and are consistent with a rigorous data collection, while the statistical analysis is innovative and appropriate. I only have very minor suggestions the authors may consider, as listed below:

Author response #1: We thank the reviewer for their positive comments regarding our manuscript and we hope that the helpful comments below have been addressed appropriately.

Comment #2: Line 63: it may be worth slightly rewording here to make absolutely clear that the papers cited are those that are 'increasingly recognising' the problem rather than being examples of the 'some cases' recognised as the problem (especially given that one of the authors' own papers is cited).

Author response #2: We agree that this sentence could lead to reader's misinterpreting the references cited as examples of cases adopting less robust statistical approaches. We have made a subtle alteration to clarify that the references cited are those that recognise the methodological and statistical challenges of these types of investigations (Introduction, page 4, lines 60-63).

Comment #3: Line 139: I expect the treadmill speed was only adjusted to achieve target relative exercise intensity in the first exercise trial (i.e. the subsequent trial would have matched the absolute intensity using the same treadmill speed as trial 1). This could be clarified.

Author response #3: Our aim was to ensure the exercise intensity for each participant was as close as possible to the target of 70% peak oxygen uptake for both exercise conditions. Therefore, the treadmill speed was adjusted slightly in both exercise conditions on the rare occasion that the relative exercise intensity was above or below the target intensity of 70% peak oxygen uptake. We have updated this sentence to clarify that the treadmill speed was adjusted during both exercise conditions if necessary (Methods: Main trials, page 8, lines 141-143).

Comment #4: It is unfortunate that there was an outlier but I feel this has been very clearly reported and thoroughly discussed such that it is not an issue.

Author response #4: We agree that it was unfortunate to have an outlier in the study and we appreciate the positive comments from the reviewer regarding the discussion of our findings.

Comment #5: Line 364-367: readers may benefit from some direction to relevant literature highlighting the potential for these factors that may alter the reported effects. Some of the authors' own papers could be cited with these lines.

Author response #5: We agree that the reader may benefit from the citation of relevant literature highlighting potential differences in appetite parameters in response to other exercise protocols or observed in other populations (e.g., females, overweight individuals). We have referenced five papers in this regard which we hope will be useful for the interested reader (Discussion, page 18, lines 397-398).

Comment #6: Table 1: missing '-1' after kg in the units for VO2max.

Author response #6: We have amended Table 1 accordingly.

Reviewer two:

Comment #1: This manuscript reports on an acute replicated cross-over study comprised of two exercise and two control acute trials to establish the interindividual appetite response to acute exercise. The popularity of personalised medicine/nutrition is growing rapidly, but to date few studies have employed a robust design to assess true interindividual responses. To my knowledge, this is the first study to employ a replicated crossover design to exercise and appetite. The manuscript is excellently written and the study has been performed under very well-controlled conditions. The statistical analyses are comprehensive and appropriate to answer the question. On that basis I would strongly recommend this manuscript for publication in Medicine and Science in Sport and Exercise on the basis of the scientific rigour which is used to answer an important, novel and topical research question. I do however, have a few points outlined below, that I feel may improve the manuscript prior to publication.

Author response #1: We thank the reviewer for their supportive comments regarding our paper and we hope that the comments raised have been addressed appropriately.

Comment #2: Could the blood sampling site (antecubital vein) influence the variability of gut hormone concentrations that were measured? It is known that both GLP-1(total) and GLP-1(7-36) concentrations are lower in venous blood compared to arterial blood (Asmar et al. 2017 Physiol Rep 5(3): e13073) presumably due to interactions with GLP-1 receptor in tissues and metabolism by DPP-IV. Could the authors comment on whether they would expect ghrelin and PYY to show anything similar in this regard? If so, then could this contribute to the variability seen? For example, the concentrations of metabolites measured in venous blood are dependent on factors such as forearm blood flow, which in turn, is altered by ambient temperature (Frayn et al. 1989 Clin Sci 76(3): 323) and it has been speculated that differences between arterialised and venous blood may depend on some characteristics of the individuals, such as forearm muscle mass/capillarisation (Edinburgh et al. 2017 Br J Nutr 117(10):1414). I do not see the sample site

as a limitation of this work, since many other studies that claim interindividual differences sample from the antecubital vein, and therefore the current study design allows the assessment of the apparent interindividual variability that is seen in these studies. It may however, be worthy of a discussion as a potential source of the variability seen.

Author response #2: We agree with the reviewer that this is an interesting point of discussion. We have not investigated differences in appetite-regulating hormone concentrations between venous and arterialised blood in any of our previous work and the literature is very limited in this regard. Previous studies in patient populations have suggested that fasting ghrelin concentrations are similar in venous and arterial blood (Goodyear et al. 2010 Mol Biol Rep 37: 3697-3701; Martin et al. 2011 Clin Invest Med 34: E82-E87); however, we are not aware of studies examining differences in PYY concentrations at the different sample sites or studies that have examined potential differences with exercise. Nevertheless, it is conceivable that the sampling site may have introduced some variability in the appetite-regulating hormone concentrations in this study and we have included the following comment in the discussion and updated the reference list accordingly:

Discussion, page 18, lines 381-390: ‘A potential source of variability in this study concerns the measurement of acylated ghrelin and total PYY concentrations from venous blood samples collected from an antecubital vein. Recent studies suggest that compared to arterialised blood, venous blood provides lower concentrations of glucagon-like peptide-1 (38) as well as lower glucose concentrations and higher insulin sensitivity (39). Although limited evidence in patient populations suggests that fasting ghrelin concentrations are comparable between venous and arterialised blood (40,41), direct comparisons of acylated ghrelin and total PYY between arterialised and venous blood after exercise has not been investigated. Nevertheless, the findings of the present study are relevant to the wider exercise and appetite regulation literature where blood sampling from an antecubital vein is commonplace for quantifying appetite-regulatory hormone concentrations.’

Comment #3: On a similar point to the sample site, where I do not believe this is a limitation, but could the exercise intensity chosen be another potential source of variability in the observed responses? At this exercise intensity some individuals may be above and some below the lactate threshold. Therefore the relative intensity for these people may be somewhat different. Secondly, if some people are exercising at an intensity above lactate threshold, then many aspects will not be in steady-state (e.g. longer slow component of VO₂ etc.). Could either of these points be relevant to the responses seen?

Author response #3: We thank the reviewer for raising this important point. The exercise intensity of 70% peak oxygen uptake was selected in order to replicate previous study designs which have consistently demonstrated changes in appetite and appetite-regulatory hormones in directions expected to suppress appetite. Although it is possible that the exercise intensity may represent a potential source of variability in the observed responses, unfortunately we do not have the data to identify whether the participants were exercising above or below their lactate threshold or to investigate further the oxygen uptake kinetics during the exercise bouts. Nevertheless, we have examined bivariate correlations between the exercise-induced change in each of the appetite

parameters with the physiological variables measured during the exercise conditions (RPE, $\dot{V}E/\dot{V}O_2$, RER and percentage of HR_{max}). This analysis revealed no significant correlations between the various appetite parameters and exercise variables ($P \geq 0.091$). Therefore, there is limited evidence based on the available data that the exercise intensity adopted in this study was associated with the variability observed in the appetite responses.

Comment #4: Line 88: was age measured to the nearest 0.1 year or were people just asked their age as a whole number? If the latter, the would it be more appropriate to report the number of decimal places to the same degree that you measured this variable at (i.e. a whole number for age)?

Author response #4: The participants provided their age as a whole number so we have amended this accordingly (Methods: Participants, page 5, line 88).

Reviewer three:

Comment #1: The study design and statistical analysis are unique to the field of exercise and appetite control. Examining the reproducibility of subjective appetite and appetite hormone responses to acute exercise is important when attempting to demonstrate robust research findings, but also when considering the application of results to the wider population. This study presents an opportunity for researchers to expand on these initial findings and contribute to work examining the effectiveness of personalised exercise prescription for weight loss. There are some minor issues that are necessary to highlight, but overall, the study is well designed and the findings are novel.

Author response #1: We thank the reviewer for their positive comments regarding our paper and we hope that we have addressed the comments below appropriately.

Comment #2: Line 95: What pre-preliminary visit controls, if any, were selected?

Author response #2: The preliminary visit was completed at a time of day that was most convenient for the participants and no special controls were implemented prior to the visit.

Comment #3: Line 96-97: Which instruments were used to conduct the screening measures?

Author response #3: Health status was assessed using the University's standard health screen questionnaire, dietary habits were assessed using the Three-Factor Eating Questionnaire (Stunkard & Messick (1985) *J Psychosom Res*, 29:71-83), and habitual physical activity was assessed using the International Physical Activity Questionnaire (Craig et al. (2003) *Med Sci Sports Exerc*, 35:1381-1395). We have updated the methods section to clarify the instruments we used to conduct the screening measures (Methods: Preliminary measurements, page 6, lines 95-100).

Comment #4: Line 129: Were the timing of the evening meals controlled?

Author response #4: Participants were asked to consume the evening meal between 19:00 and 20:00 during all four trials. We have updated the methods section to include this information (Methods: Experimental design, page 7, lines 132-134).

Comment #5: Line 137: Why was peak VO₂ chosen instead of VO₂max?

Author response #5: We determined peak oxygen uptake from an expired air sample collected in the final minute of the test using Douglas bags when participants indicated that they could only continue running for an additional 1 min. Therefore, it was not possible to ascertain whether the participants had achieved a plateau in oxygen uptake with an increase in work rate, so it is more appropriate to use the term 'peak VO₂' defined as the highest value of oxygen uptake attained on the test. In line with recent recommendations (Poole & Jones (2017) *J Appl Physiol* 122: 997-1002), we have introduced a verification stage in our subsequent studies to improve this aspect of our exercise testing which enables the verification of maximum VO₂.

Comment #6: The authors have not examined correlations between appetite sensations and appetite hormones. If possible, this analysis should be conducted, as previous research has produced equivocal findings regarding the relationship between appetite ratings and appetite hormone concentrations following exercise.

Author response #6: We thank the reviewer for this suggestion and we have calculated bivariate correlations between the pooled mean pre-to-post change in appetite-regulatory hormone concentrations and the pooled mean pre-to-post change in appetite perceptions. These results are presented in Supplementary Digital Content 2. This analysis revealed that the change in acylated ghrelin was significantly associated with hunger and prospective food consumption. In contrast, the change in PYY was not significantly associated with any of the appetite perceptions. We have updated the methods, results and discussion sections as follows:

Methods: Statistical Analyses, page 11, lines 222-224: 'Pearson's correlation coefficients were quantified between the pooled mean pre-to-post change in appetite-regulatory hormone concentrations and the pooled mean pre-to-post change in appetite perceptions across the four conditions.'

Results: Correlations, page 14, lines 284-289: 'A large positive correlation was observed between the pre-to-post change in acylated ghrelin and the change in both hunger ($r = 0.72$, 95% CI 0.33 to 0.90, $P = 0.002$) and PFC ($r = 0.63$, 95% CI 0.17 to 0.86, $P = 0.011$). There were no significant correlations between the pre-to-post change in PYY and appetite perceptions ($P \geq 0.129$) (refer to Supplemental digital content 2).'

Discussion, page 17, lines 366-367: 'and is further supported by the meaningful positive relationships observed between the pre-to-post change in acylated ghrelin and the change in hunger and PFC.'

Discussion, pages 17-18, lines 374-377: 'Indeed, the absence of significant correlations between the pre-to-post change in total PYY and appetite perceptions may reflect the notion that PYY acts synergistically with these other satiety signals to suppress appetite.'

Comment #7: Line 245: How was the outlier identified?

Author response #7: We followed the procedures recommended by Hopkins et al. (2009 *Med Sci Sports Exerc* 1:3-12) to identify the outlier for PYY. This participant exhibited a PYY response greater than 3.5 residual SDs from the mean predicted value which is the threshold advised when the sample size is less than 50. We have clarified the procedure used to identify the outlier in the results section as follows:

Results: Total PYY, page 12, lines 248-250: 'Based on the recommendations of Hopkins et al. (2009), an outlier was identified who exhibited a PYY response greater than 3.5 residual SDs from the mean predicted value (30).'

Comment #8: Lines 357-359: Despite not being a primary aim of the present study, this design did present a good opportunity to investigate these factors in more detail. The authors should suggest measurements that could be performed in future research to assess the reasons for large individual differences in appetite responses following acute bouts of exercise.

Author response #8: We thank the reviewer for this suggestion and we have identified several other appetite parameters that could be considered in future studies to provide a broader scientific understanding of the variability in appetite responses after acute exercise. We have updated the discussion as follows:

Discussion, pages 17-18, lines 372-380: 'In this regard, several other anorexigenic gut peptides are involved in the acute regulation of appetite including cholecystokinin, oxyntomodulin, pancreatic polypeptide and glucagon-like peptide-1. Indeed, the absence of significant correlations between the change in total PYY and appetite perceptions may reflect the notion that PYY acts synergistically with these other satiety signals to suppress appetite. Furthermore, appetite control is influenced by a variety of non-homeostatic factors such as neuronal responses, hedonic processes and cognitive/behavioral cues (37). Future studies should consider the aforementioned appetite parameters to provide a more holistic scientific understanding of the variability in appetite responses after acute exercise.'

Comment #9: Lines 370-372: Despite this being an appropriate reason for conducting this type of research, it is perhaps too easy to make such a statement without suggesting how research might actually enhance the effectiveness of personalised exercise interventions for weight loss.

Author response #9: We thank the reviewer for raising this important point. We agree that the reader will benefit from some additional insight on how exercise interventions could be tailored at the individual level to optimise weight management strategies. We have updated the discussion section to include the following information:

Discussion, page 18-19, lines 401-407: ‘The publication of more studies investigating individual variability in appetite responses to exercise may stimulate the development of more efficient weight management strategies by determining whether an exercise intervention is likely to be beneficial, ineffective or detrimental for different individuals. This information would help to identify individuals who may achieve more favorable appetite responses through alternative exercise and/or nutritional interventions, but further work is required to examine this chronically.’

1 **Interindividual responses of appetite to acute exercise: a replicated crossover study**

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18 **Abstract**

19 **Purpose:** Acute exercise transiently suppresses appetite, which coincides with alterations in
20 appetite-regulatory hormone concentrations. Individual variability in these responses is
21 suspected, but replicated trials are needed to quantify them robustly. We examined the
22 reproducibility of appetite and appetite-regulatory hormone responses to acute exercise and
23 quantified the individual differences in responses. **Methods:** Fifteen healthy, recreationally-
24 active men completed two control (60-min resting) and two exercise (60-min fasted treadmill
25 running at 70% peak oxygen uptake) conditions in randomised sequences. Perceived appetite
26 and circulating concentrations of acylated ghrelin and total peptide YY (PYY) were
27 measured immediately before and after the interventions. Inter-individual differences were
28 explored by correlating the two sets of response differences between exercise and control
29 conditions. Within-participant covariate-adjusted linear mixed models were used to quantify
30 participant-by-condition interactions. **Results:** Compared with control, exercise suppressed
31 mean acylated ghrelin concentrations and appetite perceptions (all ES = 0.62 to 1.47, $P <$
32 0.001), and elevated total PYY concentrations (ES = 1.49, $P <$ 0.001). For all variables, the
33 SD of the change scores was substantially greater in the exercise versus control conditions.
34 Moderate-to-large positive correlations were observed between the two sets of control-
35 adjusted exercise responses for all variables ($r = 0.54$ to 0.82 , $P \leq 0.036$). After adjusting for
36 baseline measurements, participant-by-condition interactions were present for all variables (P
37 ≤ 0.053). **Conclusion:** Our replicated cross-over study allowed, for the first time, the
38 interaction between participant and acute exercise response in appetite parameters to be
39 quantified. Even after adjustment for individual baseline measurements, participants
40 demonstrated individual differences in perceived appetite and hormone responses to acute
41 exercise bouts beyond any random within-subject variability over time.

43 **Key words**

44 Appetite; exercise; ghrelin; individual differences; peptide YY.

45 **Introduction**

46 Understanding the relationship between exercise and appetite control has direct implications
47 regarding the role of exercise in regulating energy homeostasis and weight control (1,2). It is
48 well-documented that circulating concentrations of acylated ghrelin are suppressed and
49 satiety hormones, most notably peptide YY (PYY), are elevated in response to acute bouts of
50 moderate- to high-intensity exercise (3). These hormonal fluctuations coincide with a
51 transient reduction in appetite during and immediately after exercise without stimulating
52 compensatory increases in appetite and *ad libitum* energy intake in the short term (4,5).

53 The notion of inter-individual variability in response to an intervention, within the context of
54 ‘personalised’ or ‘precision’ medicine, continues to attract significant scientific attention (6-
55 8). Whilst the majority of researchers have focussed on main effects and mean group changes,
56 some investigators have attempted to quantify the individual variability in appetite and
57 energy intake responses to acute (9-11) and chronic (12,13) exercise interventions. Some
58 researchers have classified individuals as ‘compensators’ or ‘non-compensators’ according to
59 the individual magnitude and direction of change in energy intake they observed after
60 exercise (9,10). Although the important issue of inter-individual variability has been
61 considered in exercise and appetite regulation studies, recent evidence has recognised that the
62 methodological and statistical approaches for such investigations are challenging and often
63 lacking in some cases (6,14,15).

64 One approach to quantifying “true” individual responses is via the participant-by-response
65 interaction term in a statistical model, which requires replicated intervention and comparator
66 arms with sufficient washout (16,17). Previous researchers have reported intra-class
67 coefficients to support claims that pre-to-post changes in *ad libitum* energy intake in response
68 to acute exercise are not consistent within an individual over time (11,18). Inter-individual

variability in appetite and appetite-regulatory hormone responses to repeated acute exercise exposures are suspected; however, no published studies have confirmed this notion using robust designs (the replicated cross-over) and appropriate statistical models.

Therefore, the aims of the present study were to examine the reproducibility of appetite, acylated ghrelin and total PYY responses to acute exercise bouts, and to quantify the magnitude of individual differences in responses using a replicated cross-over design. Recent insights have provided a framework for the accurate statistical analyses to quantify true inter-individual variability in exercise responses using the standard deviation (SD) of the change scores and participant-by-response interaction (6,14-17). Using these approaches, it was hypothesised that exercise-induced changes in subjective and hormonal appetite parameters would be reproducible on repeated occasions and true inter-individual variability in appetite responses to acute exercise bouts would be observed in healthy, recreationally active men.

Methods

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki (2013) and all procedures were approved by the local ethics advisory committee. All participants provided written informed consent before taking part in any aspect of the study.

Participants

Fifteen healthy, recreationally active men (mean (SD): age 23 (3) years, body mass 81.9 (11.4) kg, body mass index 24.8 (3.0) kg·m⁻², waist circumference 84.3 (6.9) cm, body fat percentage 13.1 (5.9)%, peak oxygen uptake ($\dot{V}O_2$) 54.9 (6.5) mL·kg⁻¹·min⁻¹) participated in the study. The participants' body mass was stable; ≤ 3 kg ($\leq 3.7\%$) change in the previous 3

months. Participants were non-smokers, had no history of cardiovascular or metabolic disease, and were not dieting or taking any medications.

Preliminary measurements

Before the main experimental conditions, participants attended the laboratory for a preliminary visit to complete screening questionnaires, and to undergo familiarisation, anthropometric measurements and exercise testing. Specifically, participants completed questionnaires assessing health status, food preferences, habitual physical activity (International Physical Activity Questionnaire) (19) and psychological eating tendencies (Three-Factor Eating Questionnaire) (20). Height and body mass were quantified using an electronic measuring station (Seca, Hamburg, Germany). Waist circumference was measured at the narrowest point of the torso between the lower rib margin and the iliac crest. The sum of seven skinfolds was used to estimate body density (21) and body fat percentage (22).

After familiarisation with walking and running on the treadmill (Technogym Excite Med, Cesena, Italy), participants completed two preliminary exercise tests. The first test involved a 16-min submaximal incremental treadmill protocol divided into 4×4 min stages to determine the relationship between treadmill speed and oxygen consumption. The initial running speed was set between 8 to 12 $\text{km} \cdot \text{h}^{-1}$ depending on each participant's fitness level, and the treadmill speed was increased by 1–1.5 $\text{km} \cdot \text{h}^{-1}$ at the start of each subsequent stage. Heart rate was monitored continuously using short-range telemetry (Polar A3, Kempele, Finland), and ratings of perceived exertion (RPE) (23) were assessed at the end of each stage. Expired air samples were collected into Douglas bags in the final minute of each 4 min stage. Oxygen consumption and carbon dioxide production were determined using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex 1400, East Sussex, UK), and the volume of expired air was quantified using a dry gas meter (Harvard Apparatus, Kent, UK).

After a 20-min standardised rest period, peak $\dot{V}O_2$ was measured using an incremental uphill treadmill protocol at a constant speed until the participants reached volitional fatigue. The initial incline of the treadmill was set at 3.5% which was increased by 2.5% every 3 min (24). Peak $\dot{V}O_2$ was determined from an expired air sample collected in the final minute when participants indicated that they could only continue for an additional 1 min. Heart rate and RPE were monitored throughout the tests as described previously. Data from the 16-min submaximal incremental and peak $\dot{V}O_2$ tests were used to determine the running speed required to elicit 70% of peak $\dot{V}O_2$ during the experimental exercise conditions.

Experimental design

In a replicated, cross-over experimental design, participants were randomised to different sequences of four experimental conditions: two control and two exercise (17). Each condition was separated by an interval of at least five days. Participants completed a weighed food record in the 24 h preceding the first experimental condition and were instructed to replicate this feeding pattern before each subsequent condition. Participants refrained from alcohol, caffeine, and strenuous physical activity during the same period. A standardised meal was consumed in the evening before the experimental conditions consisting of a pepperoni pizza (4891 kJ, 48% carbohydrate, 18% protein, 34% fat). Participants were instructed to consume the meal between 19:00 and 20:00, after which they consumed no food or drink except plain water until arriving at the laboratory the next morning.

Main trials

Participants arrived at the laboratory at 08:00 having fasted overnight for a minimum of 12 h. A cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein for venous blood sampling, and participants rested for 1 h (~08:00–09:00) to acclimatise to the study environment (25). During both exercise conditions, participants then

completed 60 min of fasted treadmill running at a speed predicted to elicit 70% of peak $\dot{V}O_2$. One minute expired air samples were collected and analysed every 15 minutes, and the treadmill speed was adjusted if necessary during both exercise conditions to ensure the target exercise intensity was achieved. Heart rate was monitored continuously and RPE was determined after each expired air sample was collected. The exercise energy expenditure and substrate utilisation were subsequently estimated using the equations of Frayn (26). Identical procedures were completed during both control conditions except participants rested within the laboratory for the equivalent duration.

Appetite perceptions

Ratings of perceived appetite (hunger, satisfaction, fullness and prospective food consumption (PFC)) were assessed immediately before (0 h) and after (1 h) the exercise and control interventions using 100 mm visual analogue scales (27). The scales were anchored by a descriptor at each end defining the extremes of the appetite perception being measured.

Blood sampling and biochemical analysis

Blood samples were collected in the semi-supine position immediately before (0 h) and after (1 h) the exercise and control interventions for the assessment of plasma acylated ghrelin and total PYY concentrations. Plasma acylated ghrelin concentrations were quantified from venous blood samples collected into pre-chilled 4.9 mL EDTA monovettes (Sarstedt, Leicester, UK). These monovettes contained *p*-hydroxymercuribenzoic acid to prevent the degradation of acylated ghrelin by protease and were centrifuged at 2,383 g for 10 min at 4°C (Burkard, Hertfordshire, UK). The plasma supernatant was aliquoted into a storage tube and 100 μ L of 1 M hydrochloric acid was added per milliliter of plasma. Samples were re-centrifuged at 2,383 g for 5 min at 4°C before being transferred into Eppendorf tubes and stored at -80°C for later analysis. Venous blood samples for plasma total PYY were collected

into pre-chilled 4.9 mL EDTA monovettes (Sarstedt, Leicester, UK) and centrifuged at 2,383 g for 10 min at 4°C prior to storage at -80°C. Measurements of haemoglobin and haematocrit were determined in duplicate at 0 and 1 h in all conditions to calculate the acute change in plasma volume (28).

Commercially available enzyme immunoassays were used to determine the plasma concentrations of acylated ghrelin (SPI BIO, Montigney le Bretonneux, France) and total PYY (Millipore, Watford, UK). All samples were analysed in duplicate. To eliminate inter-assay variation, samples for each participant were analysed in the same run. The within-batch coefficients of variation for acylated ghrelin and total PYY concentrations were 4.1% and 3.6%, respectively.

Statistical analyses

Data were analysed using the IBM SPSS Statistics software for Windows version 23.0 (IBM Corporation, New York, USA) and the PROC MIXED procedure in *SAS OnDemand for Academics* (https://www.sas.com/en_us/software/on-demand-for-academics.html). The presence of inter-individual differences in acylated ghrelin, total PYY and perceived appetite responses to acute exercise bouts were examined according to three recently-reported analytical approaches (6,16,17):

(i) Pearson's correlation coefficients were quantified between the exercise and control pre-to-post (0 to 1 h) change scores for each appetite parameter on the two occasions (17). The first exercise bout in any participant's sequence was paired to the first control bout in the same individual's sequence. Differences between these trials were correlated with the second exercise-control condition differences in the participant's trial sequence. Thresholds of 0.1, 0.3 and 0.5 were used to define small, moderate and large correlation coefficients, respectively (29).

(ii) The difference in SDs of the pre-to-post changes between the exercise and control conditions was calculated to represent the true individual response SD using the following equation:

$$SD_R = \sqrt{SD_E^2 - SD_C^2}$$

where SD_R is the SD of the true individual response to the exercise conditions and SD_E and SD_C are the SDs of the pre-to-post change scores for the exercise and control conditions, respectively (6,15). This estimation of the true SD for individual differences in response should be considered a “naïve estimation”, since important aspects of the experimental design, e.g. period effects, are not included. Therefore, a modelling approach to this estimation was also adopted (see iii below).

(iii) A within-participant linear mixed model was formulated to quantify any participant-by-condition interaction for each appetite parameter. Condition and period (sequence) were initially modelled as fixed effects. Senn et al. (2011) raised the question of whether the participant and participant-by-condition interaction terms should be modelled as fixed or random effects (16). Differences between these modelling approaches may exist depending on the distribution of the participant factor and the magnitude of the treatment (exercise effect). Our sample was, in clinical trial terms, relatively small and we expected the general effects of exercise to be substantial. Therefore, we modelled our data with participant and participant-by-condition terms as both fixed and random effects, and compared these results as a sensitivity analysis. When the participant-by-condition interaction was considered as a random effect, we used the SAS code supplied by Senn et al. (2011) with a modification designed to derive the true individual response variance (also estimated by approach ii) (16). This modification involved the adding of a covariate “dummy” variable we called “XVARE” (refer to the SAS code supplied in Supplemental digital content 1).

It is also relevant to explore the extent to which an individual's response depends on their status at baseline (6). Therefore, baseline status of the dependent variable was added to the various linear mixed models as a covariate. The mean differences between conditions were also quantified with this same statistical model.

We found that correction of appetite hormone concentrations for acute changes in plasma volume had a negligible influence on our findings. Therefore, the unadjusted plasma concentrations are displayed for simplicity. Absolute standardised effect sizes (ES) were calculated, with a standardised ES of 0.2 denoting the minimum important mean difference for all outcomes, 0.5 - moderate and 0.8 - large (29). To calculate the minimal clinically important difference (MCID) for individual responses, the threshold of 0.2 for interpreting standardised mean changes (29) was halved, i.e. 0.1, and multiplied by the baseline between-subject SD (6,15). Pearson's correlation coefficients were quantified between the pooled mean pre-to-post change in appetite-regulatory hormone concentrations and the pooled mean pre-to-post change in appetite perceptions across the four conditions.

Data are described as mean (SD). Mean differences and correlation coefficients are presented along with respective 95% confidence intervals (95% CI). *P*-values are expressed in exact terms apart for very low values, which are expressed as $P < 0.001$, and statistical significance was accepted as $P < 0.05$.

Results

Treadmill exercise responses

Treadmill exercise responses are displayed in Table 1. No statistically significant nor practically important differences were observed in any of the treadmill exercise responses between the two exercise sessions ($P \geq 0.13$).

Acylated ghrelin

A moderate positive correlation of 0.57 (95% CI 0.08 to 0.84, $P = 0.025$) was observed between the two sets of control-adjusted exercise responses for acylated ghrelin (Figure 1A). The within-trial SD for acylated ghrelin was substantially greater for the exercise than control conditions (Table 2). Baseline-adjusted linear mixed models for acylated ghrelin concentrations revealed a significant main effect of condition ($P < 0.001$) and a significant participant-by-condition interaction ($P < 0.001$). The mean acylated ghrelin concentration was 51 $\text{pg} \cdot \text{mL}^{-1}$ lower (95% CI -59 to -43 $\text{pg} \cdot \text{mL}^{-1}$, ES = 0.62) in the exercise versus control conditions. The magnitude of change in individual replicated mean responses after exercise for acylated ghrelin ranged from -141 to -9 $\text{pg} \cdot \text{mL}^{-1}$, with 100% ($n = 15$) of participants demonstrating a suppression beyond the MCID ($\pm 8.20 \text{ pg} \cdot \text{mL}^{-1}$) (Figure 1B).

Total PYY

A small positive correlation of 0.27 (95% CI -0.28 to 0.69, $P = 0.339$) was observed between the two sets of control-adjusted exercise responses for total PYY (Figure 2A). Based on the recommendations of Hopkins et al. (2009), an outlier was identified who exhibited a PYY response greater than 3.5 residual SDs from the mean predicted value (30). After removal of the outlier, the correlation for total PYY increased to 0.71 and became significant (95% CI 0.31 to 0.90, $P = 0.003$) (Figure 2B). The within-trial SD for total PYY was substantially greater for the exercise than control conditions (Table 2). Baseline-adjusted linear mixed models for total PYY concentrations revealed a significant main effect of condition ($P < 0.001$) and a significant participant-by-condition interaction ($P = 0.012$). The mean total PYY

concentration was 56 pg·mL⁻¹ higher (95% CI 44 to 68 pg·mL⁻¹, ES = 1.49) in the exercise versus control conditions. The magnitude of change in individual replicated mean responses after exercise for total PYY ranged from 3 to 112 pg·mL⁻¹, with 93% ($n = 14$) of participants demonstrating an increase beyond the MCID (± 3.75 pg·mL⁻¹) (Figure 2C).

Appetite ratings

Moderate-to-large positive correlations were observed between the two sets of control-adjusted exercise responses for hunger ($r = 0.82$, 95% CI 0.53 to 0.94, $P < 0.001$), satisfaction ($r = 0.74$, 95% CI 0.37 to 0.91, $P = 0.002$), fullness ($r = 0.55$, 95% CI 0.05 to 0.83, $P = 0.035$) and PFC ($r = 0.54$, 95% CI 0.04 to 0.82, $P = 0.036$) (Figure 3). The within-trial SD was substantially greater for the exercise than control conditions for hunger, satisfaction, fullness and PFC (Table 2).

Baseline-adjusted linear mixed models for all ratings of perceived appetite revealed a main effect of condition ($P < 0.001$) and participant-by-condition interactions ($P \leq 0.053$). The main effect of condition identified suppressed appetite in the exercise compared with control conditions. The mean ratings of hunger and PFC were 26 mm (95% CI -29 to -22 mm, ES = 1.47) and 19 mm (95% CI -25 to -13 mm, ES = 1.05) lower in the exercise versus control conditions, respectively. The mean ratings of satisfaction and fullness were 15 mm (95% CI 11 to 20 mm, ES = 0.95) and 14 mm (95% CI 8 to 21 mm, ES = 0.88) higher in the exercise versus control conditions, respectively. The magnitude of change in individual replicated mean responses after exercise ranged from -65 to 10 mm for hunger, -13 to 72 mm for satisfaction, -23 to 89 mm for fullness and -96 to 7 mm for PFC. Ninety-three percent ($n = 14$) of participants demonstrated a response beyond the MCID for hunger (± 1.76 mm; 13% above, 80% below) and satisfaction (± 1.62 mm; 60% above, 33% below), 87% ($n = 13$) for

fullness (± 1.64 mm; 53% above, 33% below) and 100% ($n = 15$) for PFC (± 1.82 mm; 33% above, 67% below) (Figure 4).

A sensitivity analysis with the participant factor entered into the statistical model as a random, rather than a fixed, effect also resulted in participant-by-condition interactions for all appetite parameters (Table 2, $P = 0.013$ – 0.077).

Correlations

A large positive correlation was observed between the pre-to-post change in acylated ghrelin and the change in both hunger ($r = 0.72$, 95% CI 0.33 to 0.90, $P = 0.002$) and PFC ($r = 0.63$, 95% CI 0.17 to 0.86, $P = 0.011$). There were no significant correlations between the pre-to-post change in PYY and appetite perceptions ($P \geq 0.129$) (refer to Supplemental digital content 2).

Discussion

The primary finding from our replicated cross-over trial of appetite responses to exercise was that true inter-individual variability exists in the appetite, acylated ghrelin and total PYY responses to acute exercise bouts beyond any measurement error and random within-subject variability over time. A further finding was the moderate-to-large positive correlations observed between the exercise and control pre-to-post change scores on two occasions, indicating good reproducibility for exercise-induced changes in appetite parameters.

Our study supports previous literature by confirming the appetite suppressing effect of acute exercise (3,5). In this regard, the grand mean changes at the sample level indicated a suppression of acylated ghrelin and perceived appetite, and an increase in total PYY after the exercise session. The correlation coefficients quantified between the exercise and control pre-to-post change scores on the two pairs of conditions were positive, significant and moderate-

to-large for perceived appetite and acylated ghrelin. Although the correlation for total PYY was small and non-significant, closer examination of the change scores revealed that one participant presented two very opposite responses to exercise. Specifically, the change score between the first pair of trials indicated a suppression in total PYY ($-34 \text{ pg} \cdot \text{mL}^{-1}$) and the second pair of trials showed a very strong increase in total PYY levels ($146 \text{ pg} \cdot \text{mL}^{-1}$) (Figure 2A, 2C). The reason for this disparity is unclear and removal of this apparent outlier resulted in a larger correlation of similar magnitude to the other appetite-related outcomes measured in our study. Overall, responses to exercise were similar on repeated occasions, providing evidence to support the reproducibility of changes in appetite parameters after acute exercise.

While no previous researchers have quantified the reproducibility of perceived appetite or appetite-regulatory hormone responses to acute exercise, the reproducibility of post-exercise energy intake has received more attention (11,18,31). Specifically, Laan et al. (31) reported good reproducibility for ad libitum energy intake after duplicate aerobic exercise, resistance exercise and resting control conditions in young, active adults (31). However, the difference in ad libitum energy intake between the exercise and control conditions was not calculated in the study by Laan et al. (31). Therefore, it can be said that within-subject variations were not taken into account and the possibility of the observed responses to exercise being exclusively due to measurement errors and random variability cannot be excluded (6,15). Although energy intake appears reproducible when considering repeated resting and exercise conditions in isolation (11,31), the reproducibility of the difference in ad libitum energy intake between exercise and control interventions appears low when assessed with the use of intra-class coefficients (11,18).

Alongside the good reproducibility of appetite responses to acute exercise, our data show that individuals differ in the general magnitude of this response (the mean of the replicated trials, Figures 1B, 2C and 4). A statistically significant participant-by-condition interaction was

observed for all appetite parameters, even after adjusting for baseline values. Although previous studies have reported individual variability in perceived appetite and energy intake responses to acute exercise in healthy (9) and overweight and obese women (10), this variability was estimated using a single pair of trials, i.e. one control and one exercise condition. Repeated administrations of treatment in a cross-over fashion with a comparator arm (control condition) are required to assess individual variability in response to short-term or acute interventions from the participant-by-condition interaction term (15). We are not aware of previous studies assessing individual variability in appetite and appetite-regulatory hormone responses to acute exercise using a replicated cross-over design and the statistical methods employed in the present study.

The SD of the change scores is a good indication of individual variability in the responses to an intervention. If the SD of the change scores does not differ substantially between control and intervention conditions, the change originated by the intervention could be explained by random within-subject variation and measurement error (6,15). The true individual response SD (using both estimates 1 and 2) was relatively large compared with the mean response for all appetite-related variables measured in this study (Table 2). For example, while the mean unadjusted exercise response (versus control change) for acylated ghrelin was approximately $47 \text{ pg} \cdot \text{mL}^{-1}$, the true individual response SD was approximately $\pm 30 \text{ pg} \cdot \text{mL}^{-1}$ (Table 2). This SD indicates the presence of substantial true inter-individual differences in the acylated ghrelin response to exercise; this interpretation also applies to the other appetite parameters we assessed.

Furthermore, we also highlight that the vast majority of participants showed appetite responses that exceeded the MCID we selected. Therefore, very few participants were identified as “non-responders”, but some were “very large responders” while others were “small responders” according to the magnitude of change in acylated ghrelin, total PYY and

appetite perceptions after single bouts of exercise (Figures 1B, 2C, 4). Specifically, all participants demonstrated replicated mean responses beyond the MCID for circulating acylated ghrelin indicating an exercise-induced suppression of this hormone, and 93% of participants experienced an increase in circulating total PYY beyond the MCID. The direction of the replicated mean responses was more variable for the perceived appetite ratings. Of the participants that demonstrated replicated mean responses beyond the MCID, 53–80% of participants reported suppressed appetite after exercise (i.e., lower hunger and PFC, higher satisfaction and fullness), whereas 13–33% of participants reported higher perceived appetite after exercise (i.e., higher hunger and PFC, lower satisfaction and fullness).

Although some studies report concomitant changes in appetite-regulatory hormones and appetite perceptions in response to acute exercise at the group level (32,33), exercise-induced changes in these parameters do not always occur simultaneously (34-36). The present study extends these findings by demonstrating that the majority of participants exhibited corresponding exercise-induced changes in acylated ghrelin, total PYY and appetite perceptions, and is further supported by the meaningful positive relationships observed between the pre-to-post change in acylated ghrelin and the change in hunger and PFC.

However, some participants demonstrated divergent subjective and hormonal appetite responses to exercise. It is well established that appetite regulation is a complex process involving the interaction of many physiological and psychological factors (1). Therefore, perceived appetite in some participants could have been more strongly affected by other variables not assessed in the present study. In this regard, several other anorexigenic gut peptides are involved in the acute regulation of appetite including cholecystokinin, oxyntomodulin, pancreatic polypeptide and glucagon-like peptide-1. Indeed, the absence of significant correlations between the pre-to-post change in total PYY and appetite perceptions may reflect the notion that PYY acts synergistically with these other satiety signals to

378 suppress appetite. Furthermore, appetite control is influenced by a variety of non-homeostatic
379 factors such as neuronal responses, hedonic processes and cognitive/behavioural cues (37).
380 Future studies should consider the aforementioned appetite parameters to provide a more
381 holistic scientific understanding of the variability in appetite responses after acute exercise.

382 A potential source of variability in this study concerns the measurement of acylated ghrelin
383 and total PYY concentrations from venous blood samples collected from an antecubital vein.
384 Recent studies suggest that compared to arterialised blood, venous blood provides lower
385 concentrations of glucagon-like peptide-1 (38) as well as lower glucose concentrations and
386 higher insulin sensitivity (39). Although limited evidence in patient populations suggests that
387 fasting ghrelin concentrations are comparable between venous and arterialised blood (40,41),
388 direct comparisons of acylated ghrelin and total PYY between arterialised and venous blood
389 after exercise has not been investigated. Nevertheless, the findings of the present study are
390 relevant to the wider exercise and appetite regulation literature where blood sampling from an
391 antecubital vein is commonplace for quantifying appetite-regulatory hormone concentrations.

392 The strengths of our study include the replicated cross-over design and the use of recently
393 published robust statistical analyses for individual variability quantification. Moreover, the
394 detailed standardisation protocol followed by all participants during the 24 h preceding each
395 laboratory visit and the precise replication of the exercise sessions add credibility to our
396 results. However, it should be highlighted that our results cannot be generalized to other
397 populations such as females, overweight or obese, and older individuals who may present
398 different results (42,43). It is also possible that different exercise modes, intensities, or
399 session durations would elicit different responses (5,34,44). Therefore, further research is
400 needed to assess the reproducibility and individual variability of exercise-induced changes in
401 appetite-regulatory hormones and appetite perceptions in other populations and with different
402 exercise protocols. The publication of more studies investigating individual variability in

appetite responses to exercise may stimulate the development of more efficient weight management strategies by determining whether an exercise intervention is likely to be beneficial, ineffective or detrimental for different individuals. This information would help to identify individuals who may achieve more favourable appetite responses through alternative exercise and/or nutritional interventions, but further work is required to examine this chronically.

In conclusion, healthy, young men exhibited reproducible appetite responses to acute exercise, and true individual variability exists in acylated ghrelin, total PYY and perceived appetite responses over and above any random within-subject variability and measurement error. Individual variability in appetite responses to acute exercise needs to be considered when interpreting study results so that misleading conclusions can be avoided.

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Conflicts of interest

The authors declare no conflict of interest. The authors declare that the results of the study
are presented clearly, honestly, and without fabrication, falsification, or inappropriate data
manipulation and do not constitute endorsement by the American College of Sports Medicine.

Supplemental digital content

Supplemental digital content 1.docx

Supplemental digital content 2.docx

Figure legends

Figure 1. (A) Relationship between exercise and control pre-to-post (0 to 1 h) change scores on the two occasions for acylated ghrelin. 'Response 1' corresponds to the first pair of conditions (exercise 1 minus control 1) and 'Response 2' to the second pair of conditions (exercise 2 minus control 2). Dashed lines represent the mean responses. (B) Individual changes in acylated ghrelin between the exercise and control conditions (exercise minus control). Black circles (●) indicate pre-to-post change scores for 'response 1' and 'response 2' for each participant. Grey lines (—) represent each participants' replicated mean response. Dashed lines indicate the standardised minimal clinically important difference calculated as 0.1 multiplied by the baseline between-subject SD (6).

Figure 2. Relationship between exercise and control pre-to-post (0 to 1 h) change scores on the two occasions for total PYY before (A) and after (B) the removal of a substantial outlier. 'Response 1' corresponds to the first pair of conditions (exercise 1 minus control 1) and 'Response 2' to the second pair of conditions (exercise 2 minus control 2). Dashed lines represent the mean responses. (C) Individual changes in total PYY between the exercise and control conditions (exercise minus control). Black circles (●) indicate pre-to-post change scores for 'response 1' and 'response 2' for each participant. Grey lines (—) represent each participants' replicated mean response. Dashed lines indicate the standardised minimal clinically important difference calculated as 0.1 multiplied by the baseline between-subject SD (6).

562 **Figure 3.** Relationship between exercise and control pre-to-post (0 to 1 h) change scores on
563 the two occasions for (A) hunger, (B) satisfaction, (C) fullness, and (D) prospective food
564 consumption (PFC). 'Response 1' corresponds to the first pair of conditions (exercise 1 minus
565 control 1) and 'Response 2' to the second pair of conditions (exercise 2 minus control 2).
566 Dashed lines represent the mean responses.

567

568 **Figure 4.** Individual changes in each perceived appetite ratings between the exercise and
569 control conditions (exercise minus control): (A) hunger, (B) satisfaction, (C) fullness, (D)
570 prospective food consumption (PFC). Black circles (●) indicate pre-to-post change scores for
571 'response 1' and 'response 2' for each participant. Grey lines (—) represent each participants'
572 replicated mean response. Dashed lines indicate the standardised minimal clinically important
573 difference calculated as 0.1 multiplied by the baseline between-subject SD (6).

Table 1 The various responses during the treadmill exercise for the two exercise conditions.

Variable	Exercise	Exercise	95% CI*	ES
	condition 1	condition 2		
Oxygen uptake (mL·kg ⁻¹ ·min ⁻¹)	38.9 (5.1)	38.5 (4.9)	-4.2 to 3.3	0.09
% peak oxygen uptake	71 (3)	70 (3)	-2 to 0.3	0.31
Heart rate (beats·min ⁻¹)	176 (10)	176 (13)	-5 to 4	0.04
Rating of perceived exertion	15 (2)	15 (2)	-1 to 0.2	0.13
Respiratory exchange ratio	0.91 (0.03)	0.92 (0.04)	-0.01 to 0.02	0.21
Fat oxidation (g)	29 (12)	26 (14)	-7 to 2	0.22
Carbohydrate oxidation (g)	159 (29)	164 (36)	-6 to 15	0.13
Net energy expenditure (kJ)	3473 (551)	3433 (532)	-104 to 23	0.08

Values are mean (SD). *95% confidence interval for the mean absolute difference between exercise conditions. ES - standardised (to between-subjects SD) effect size.

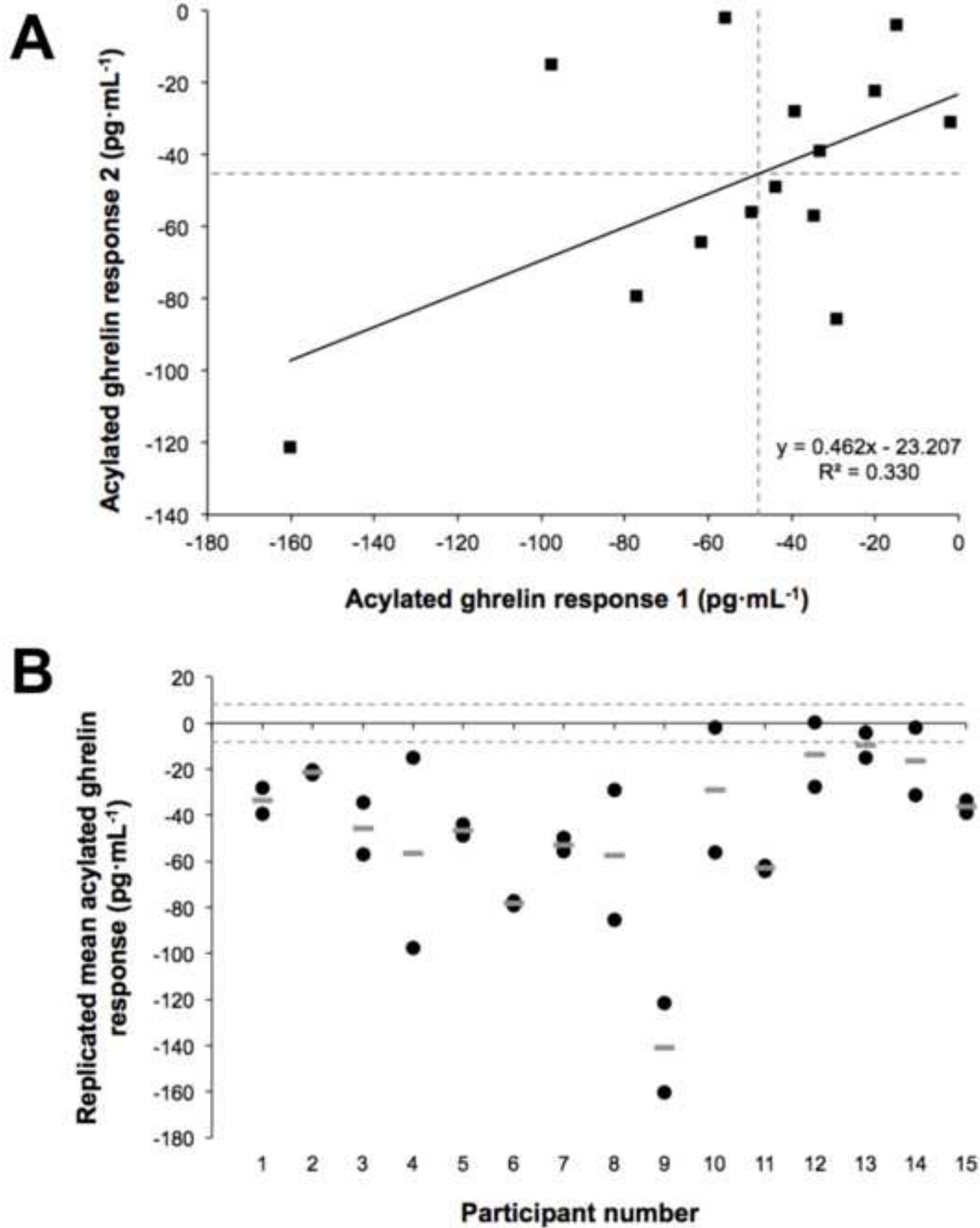
Table 2 Unadjusted mean and standard deviations (SD) of the pre-to-post change scores for the exercise and control conditions and the true individual differences SD.

Variable	Exercise change Mean (SD)	Control change Mean (SD)	Estimate 1 ^a	Estimate 2 ^b	<i>P</i> -value
			Individual	Individual	
			differences SD	differences SD (SE)	
Acylated ghrelin (pg·mL ⁻¹)	-41.9 (33.1)	4.8 (13.0)	30.4	30.9 (19.7)	0.014
Total PYY (pg·mL ⁻¹)	40.7 (35.5)	-10.7 (23.1)	27.0	25.7 (19.3)	0.077
Hunger (mm)	-13.6 (26.8)	10.5 (7.5)	25.7	24.5 (15.5)	0.013
Satisfaction (mm)	6.5 (25.1)	-7.7 (8.9)	23.5	23.2 (14.8)	0.015
Fullness (mm)	3.6 (34.8)	-8.3 (9.8)	33.4	31.6 (20.1)	0.013
Prospective food consumption (mm)	-9.9 (27.7)	7.7 (9.6)	26.0	23.7 (15.5)	0.019

^a Estimate 1: Individual differences SD estimated using $SD_R = \sqrt{SD_E^2 - SD_C^2}$ where SD_R is the SD of the true individual response, and SD_E and SD_C are the SDs of the pre-to-post change scores for the exercise and control conditions, respectively (6,15).

^b Estimate 2: Individual differences SD estimated using a random effects statistical model based on Senn et al. (16). The SD was derived from the SAS model participant-by-condition interaction term (as a random effect). The *P*-value shown is also for this interaction term.

SE, standard error.



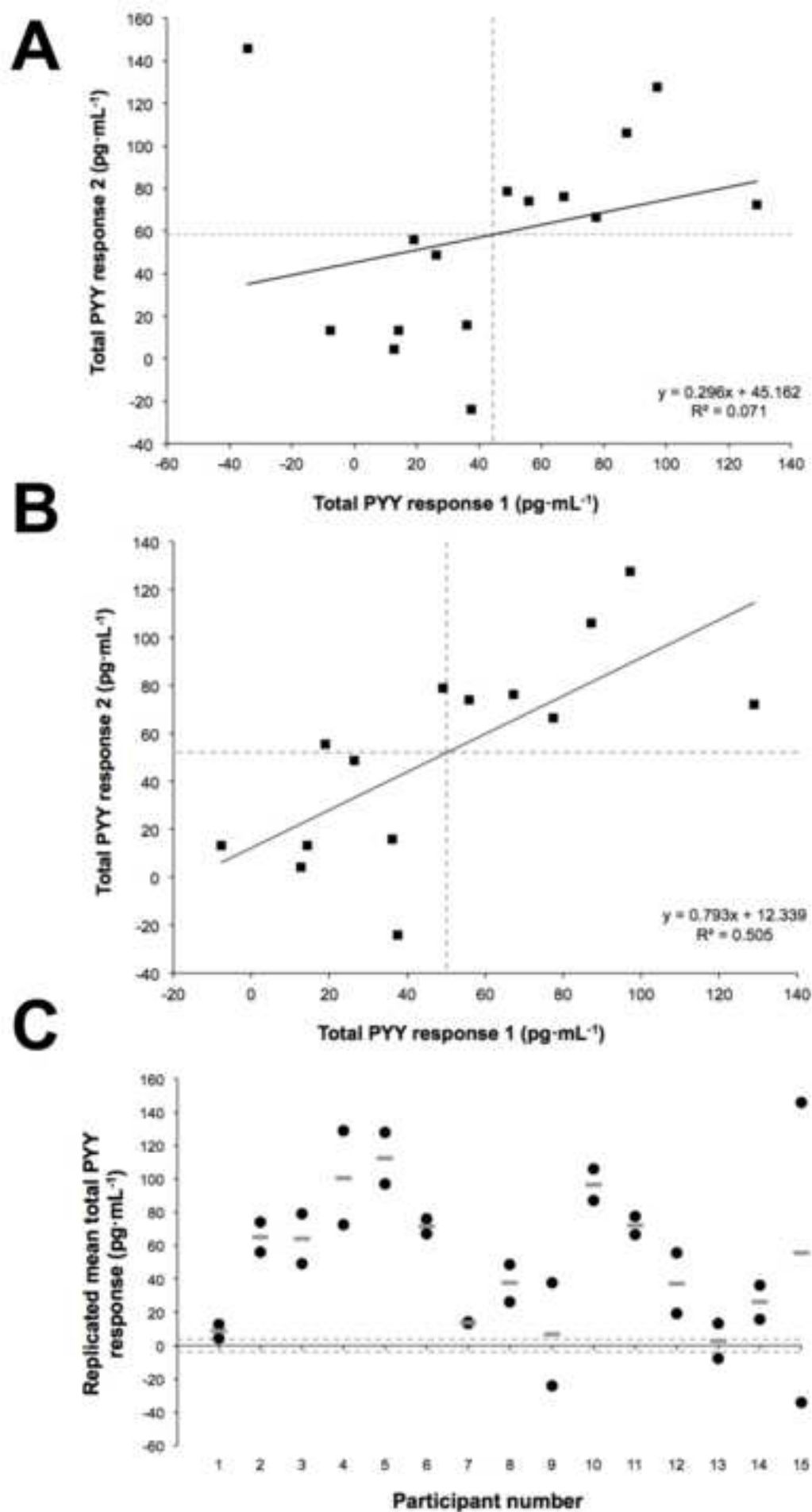


Figure 3

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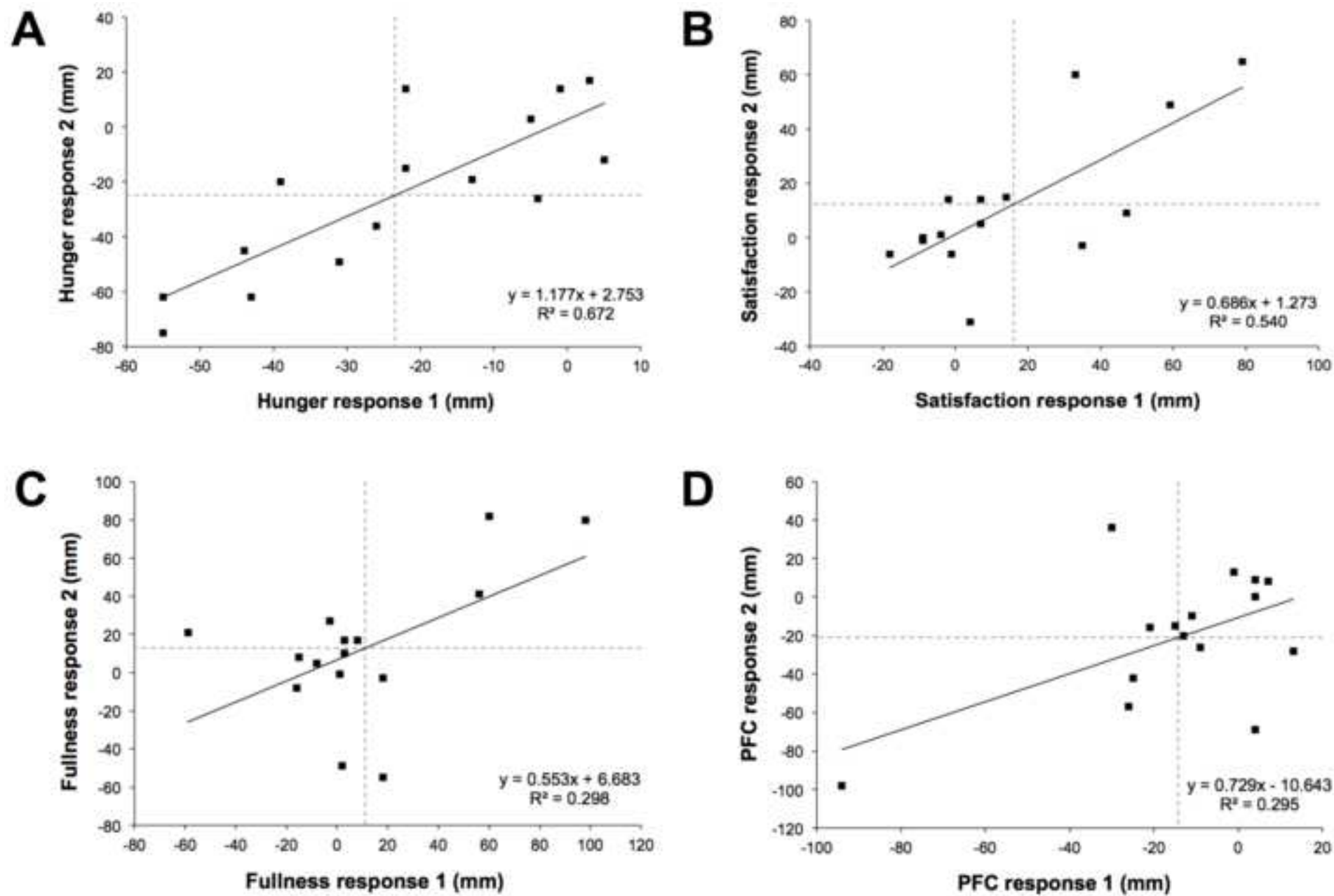
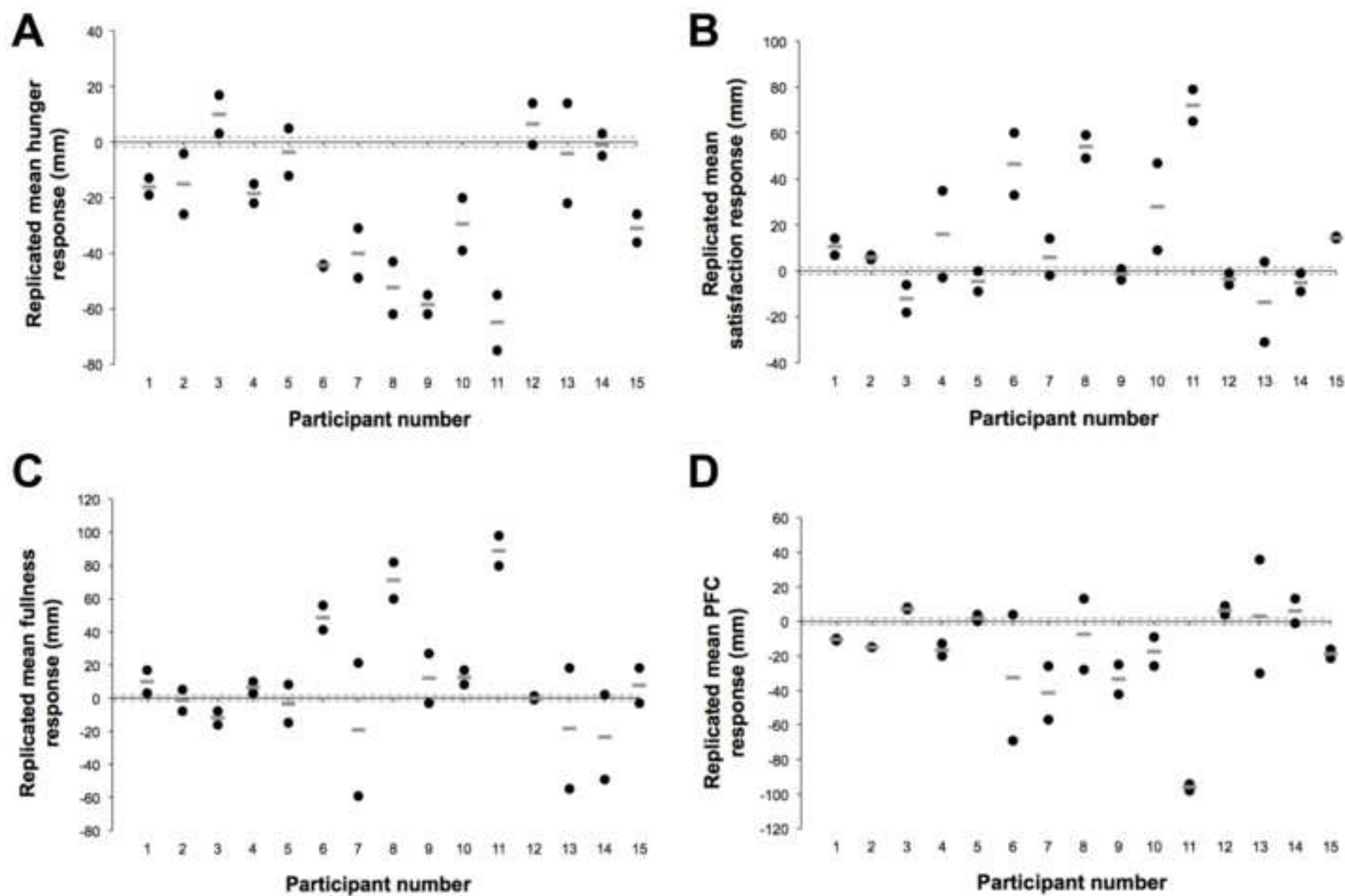
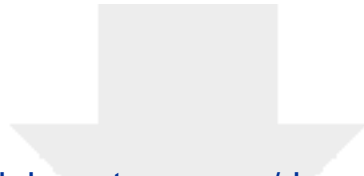


Figure 4

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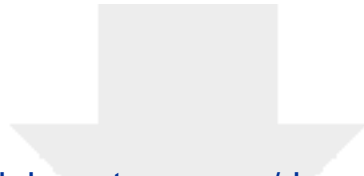


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